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Wheat starch swelling, gelatinization and pasting: Effects of enzymatic modification of wheat endogenous lipids

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ABSTRACT

Starch is widely used in food industry because of its unique gelling, thickening and stabilizing capacities. These characteristics are impacted by added surfactants. However, less is known about whether and, if so, how wheat endogenous lipids impact the swelling behaviour of starch granules. We here used three different lipases (Lecitase Ultra, Lipopan F and Lipolase) with known impact on the endogenous lipid composition and two surfactants (diacetyl tartaric esters of mono- and diacylglycerols and sodium stearoyl lactylate) for studying the impact of (endogenous) lipids on starch rheology and carbohydrate leaching. The study revealed that although amylose-lipid inclusion complex formation affects wheat starch swelling and carbohydrate leaching, there is no causal relation between the two latter phenomena. Both their location and type affect the impact of lipids on starch swelling. Next to the complex forming ability of lipid(-like) components, their ability to shield starch granules from water by forming lipophilic layers also affects starch granule swelling because it delays water absorption and increases starch granule rigidity.

1. Introduction

Starch is used in a broad range of food recipes because of its unique gelling, thickening and stabilizing capacities. It occurs as water-insoluble semi-crystalline granules (Eliasson & Gudmundsson, 1996; French, 1973) which contain amylose (AM) and amylopectin (AP) (respectively 22–28% and 78–72% for wheat starch) (Lineback, 1984; Zobel, 1988). When starch granules are heated in water, they absorb water and swell. While below the gelatinization temperature this process is reversible, above this temperature irreversible changes result in loss of crystallinity and granule disruption (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988; Delcour & Hoseney, 2010), the extent of which amongst others depends on the water level and the presence of components such as sugar or salt (Lelievre, 1976; Wootton & Bamunuarachchi, 1980). Gelatinization and pasting involve (i) granule swelling, (ii) leaching of carbohydrate material (mainly AM), (iii) the formation of a three-dimensional starch network of leached material as well as (iv) interactions between granule remnants and the leached material (Atwell et al., 1988). Upon cooling, a gel is formed because leached AM crystallizes into double helices in the continuous phase. The latter phenomenon is referred to as gelation (Delcour & Hoseney, 2010).

In wheat flour, starch granules are surrounded by amyloplast membrane remnants (Hargin & Morrison, 1980) which originate from lipid bilayer membranes that surround the amyloplasts in which starch is synthesized and stored during kernel development (Bechtel & Wilson, 2003). The bilayer membranes mainly consist of galacto- and phospholipids. However, during seed desiccation, amyloplast lipid bilayer membranes are (at least partially) degraded (Tan & Morrison, 1979). The presence of lipids at the starch granule surface may well impact the behaviour of AM and AP prior to and during gelatinisation and pasting. Within this context, the impact of surfactants commonly used in bread making (Eliasson, 1985; Gudmundsson & Eliasson, 1990; Krog, 1973; Van Steertegem, Pareyt, Brijs, & Delcour, 2013) on starch pasting has been studied profoundly, and was mainly attributed to formation of AM-lipid inclusion (AM-L) complexes. However, not much is

Abbreviations: AM, amylose; AM-L, amylose-lipid inclusion; AP, amylopectin; C*, close packing concentration; CHL, carbohydrate leaching; DATEM, diacetyl tartaric esters of mono- and diacylglycerols; DSC, differential scanning calorimetry; EP, enzyme protein; FFA, free fatty acids; MAG, monoacylglycerols; RVA, Rapid Visco Analyser; SSL, sodium stearoyl lactylate; SP, swelling power; TAG, triacylglycerols.

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known about the impact of wheat flour endogenous lipids on gelatinization and pasting of starch in wheat flour. It is also unclear whether the lipids associated with the starch granule surface induce effects similar to those of lipids not occurring at the starch granule surface, *e.g.* those present in spherosomes or associated with gluten in flour.

To study whether selective alteration of the endogenous lipid population affects wheat flour behaviour in a starch gelatinization, pasting and gelation cycle, three different lipases with known impact on the lipid composition, *i.e.* Lecitase Ultra, Lipopan F and Lipolase (Gerits, Pareyt, & Delcour, 2014) were applied. Lipases selectively alter endogenous lipids and thereby increase the amount of surfactants present without altering other flour constituents. An overview of the action mechanisms and cleavage sites of different lipases is given in Gerits, Pareyt, Decamps, and Delcour (2014). More in particular, Lecitase Ultra and Lipopan F mainly hydrolyse galactolipids and to a lesser extent phospholipids in the bound lipid fraction, thereby increasing the level of 'lyso' lipids and free fatty acids (FFA). Lipolase mainly acts on triacylglycerols (TAG) in the free lipid fraction, and thereby releases FFA. Free and the bound lipid fractions are defined based on their sequential extraction from wheat flour or dough with hexane (free lipids) and water saturated butanol (bound lipids) (Gerits, Pareyt, & Delcour, 2014). The changes in Rapid Visco Analyser (RVA) profiles and differential scanning calorimetry (DSC) AP crystallite thermograms upon lipase use were compared to those observed upon addition of surfactants [diacetyl tartaric esters of mono- and diacylglycerols (DATEM) and sodium stearoyl lactylate (SSL)]. Both surfactants are able to form lamellar mesophases in water at room temperature (Krog, 1981). The swelling power (SP) and carbohydrate leaching (CHL) of starch in wheat flour either supplemented with the lipases or surfactants or not, were analysed as well.

2. Materials & methods

2.1. Materials

Grains from soft wheat cultivar Claire were from Limagrain (Rothwell, UK) and conditioned to 16.0% moisture before milling with a Bühler (Uzwil, Switzerland) MLU-202 laboratory mill using the milling diagram outlined in Delcour, Vanhamel, and De Geest (1989). The milling yield of straight grade flour was 69.4%, and its moisture and protein contents were respectively 14.8% and 10.3% [on dry matter basis]. The latter were determined with AACCI Approved Method 44-19.01 (AACCI, 1999) and an adaptation of the AOAC Official Method (AOAC, 1995) to an automated Dumas protein analysis system (EAS Vario Max CN, Elt, Gouda, The Netherlands) with 5.7 as nitrogen to protein conversion factor, respectively. Its lipid composition is depicted in Gerits, Pareyt, and Delcour (2013). The enzyme preparations were kindly donated by Novozymes (Bagsvaerd, Denmark). Lipopan F, a *Fusarium oxysporum* enzyme preparation, is used in bread making as a source of lipase and phospholipase activities. Lecitase Ultra is a phospholipase used in edible oil degumming. It is the result of combining homologous genes encoding *Thermomyces lanuginosus* lipase and *Fusarium oxysorum* phospholipase (De Maria, Vind, Oxenboll, Svendsen, & Patkar, 2007). Lipolase, a recombinant *T. lanuginosus* lipase is used in detergent systems (Aravindan, Anbumathi, & Viruthagiri, 2007). Lecitase Ultra, Lipopan F and Lipolase had lipase activities against *p*-nitrophenyl palmitate of 0.15 units (U), 56.50 U and 0.12 U, respectively, with one U being the amount (in μmole) of *p*-nitrophenol released per minute and per mg enzyme under the conditions of the assay (Gerits, Pareyt, Decamps, et al., 2014; Gerits, Pareyt, & Delcour, 2014). How the lipases affect wheat lipid composition during bread making was studied in Gerits, Pareyt, and Delcour (2014). DATEM and SSL were from Puratos (Groot-Bijgaarden, Belgium). All solvents used were from VWR (Haasrode, Belgium) unless specified otherwise and of at least analytical grade.

2.2. Methods

2.2.1. Dough making

Dough was made according to Shogren and Finney (1984) on 10 g scale but without shortening. Flour (10.0 g on a 14.0% moisture base), water, sugar (6.0% on flour basis), compressed yeast (5.3% on flour basis) and salt (1.5% on flour basis) were mixed in a 10 g pin mixer (National Manufacturing, Lincoln, NE). The water added and the optimal mixing time were determined by Mixograph analysis (National Manufacturing) according to AACCI Approved Method 54-40.02 (AACCI, 1999) and were respectively 5.1 ml and 150 s. Lipases (Lecitase Ultra, Lipopan F or Lipolase), SSL or DATEM were included in the recipe in levels of 0.5 and 5 mg enzyme protein (EP) lipase/kg flour and 0.5% surfactant (dry powder, on flour basis), respectively.

2.2.2. Differential scanning calorimetry

DSC analysis was performed with a Q1000 DSC (TA instruments, New Castle, DE, USA). At least three fermented (120 min at 30 °C, to allow for enzyme activity) dough samples (4.0–7.0 mg) were accurately weighed into aluminium pans (Perkin Elmer, Waltman, MA, USA). Deionized water was added in a ratio of 1:3 w/w sample dry matter: water. The pans were hermetically sealed and equilibrated at 0 °C before heating to 140 °C at 4 °C/min (together with an empty reference pan). The system was calibrated with indium. Onset and conclusion temperatures and enthalpies (J/g sample) of AP crystallite melting and dissociation of the AM-L complexes [96–100 °C for amorphous and 105–125 °C for semi-crystalline complexes (Karkalas, Ma, Morrison, & Pethrick, 1995)] of fermented control dough or fermented dough containing lipase (0.5 and 5.0 mg EP/kg flour) or surfactant (0.5% on flour basis) were calculated with TA Instruments Universal Analysis software.

2.2.3. Rapid visco-analysis

Swelling, pasting and gelation of starch in wheat flour was studied with a Rapid Visco Analyser (RVA-4D, Newport Scientific, Sydney, Australia). Flour suspensions [12.0% dm, *i.e.* above the close packing concentration (C*, *cfr. infra*), in deionized water (total weight 25.0 g)] were prepared in duplicate with or without added lipases (0.5, 1.0 and 5.0 mg EP/kg flour) or surfactants [0.5 and 1.5% (on flour basis) DATEM or SSL]. The suspensions were subjected to a time–temperature profile which consisted of an incubation step of 10 min at 30 °C (to allow for lipase activity), a heating step to 95 °C at 3.25 °C/min, an isothermal step at 95 °C for 5 min, a cooling step to 50 °C at 4.5 °C/min and a final isothermal step at 50 °C for 10 min. The stirring speed was 160 rpm.

2.2.4. Determination of swelling power and carbohydrate leaching

Swelling power (SP) of wheat flour at three different temperatures and with or without addition of 5.0 mg EP Lecitase Ultra or Lipolase/kg flour or 1.5% (on flour basis) SSL or DATEM were determined according to Eerlingen, Jacobs, Block, and Delcour (1997). Prior to analysis, wheat flour suspensions (100 mg in 9.0 ml deionized water) were incubated at room temperature for 120 min to allow for lipase action. Thereafter, the suspensions were heated at 45 °C, 75 °C or 95 °C for 30 min, with shaking every 5 min. Samples were allowed to cool for 5 min and centrifuged for 30 min at 1000 *g* at 20 °C. Analyses were performed in triplicate. Carbohydrate leaching (CHL) was determined on the supernatant as in Dubois, Gilles, Hamilton, Rebers, and Smith (1956) and expressed as a percentage of total dry matter starch. SP and the C*,

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which is defined as the concentration at which the amount of water present is that which the flour can take up during cooking (Derycke et al., 2005), were calculated as follows:

$$SP = \frac{sediment\ weight\ \times\ 100}{(dm\ flour\ weight) \times (100 - \%CHL)}$$

$$C^* = \frac{dm\ flour\ weight\ \times 100}{sediment\ weight}$$

2.2.5. Statistical analyses

Statistical analyses were performed with the Statistical Analysis System software 9.3 (SAS Institute, Cary, NC, USA). For several variables, it was verified whether mean values, based on at least three individual measurements, significantly differed (significance level $\alpha = 0.05$, ANOVA analysis).

3. Results & discussion

3.1. Differential scanning calorimetry

DSC analyses revealed significant differences neither in onset and conclusion temperatures nor in enthalpies of starch gelatinization between control dough and dough samples with added lipases, SSL or DATEM, as recently demonstrated in Gerits, Pareyt, Masure, and Delcour (2015). AP crystal melting is a starch granule internal event (Pauly, Pareyt, De Brier, Fierens, & Delcour, 2012). Hence, it is affected neither by conversion of extragranular wheat lipids nor by DATEM or SSL addition.

In contrast, all lipases tested significantly and to a similar extent increased the enthalpy of the endotherm associated with dissociation of amorphous (Type I) AM-L complexes (Fig. 1). Semi-crystalline (Type II) AM-L complexes were not detected. As demonstrated in Gerits, Pareyt, and Delcour (2014), Lecitase Ultra and Lipopan F lipase activities increase the levels of FFA in the free lipid fraction and that of 'lyso' lipids in the bound lipid fraction. Lipolase, in contrast, mainly converts TAG to FFA in the free lipid fraction. Taken together, under the conditions tested here and based on the action mechanisms of the different lipases, the formation of AM-L complexes depends neither on the type (*i.e.* mainly polar lipids in the case of Lecitase Ultra and Lipopan F *vs.* mainly non-polar lipids in the case of Lipolase) nor on the location (*i.e.* in the gluten network or at the starch granule surface, as is the case for Lecitase Ultra and Lipopan F, or in spherosomes in the case of Lipolase) of the lipids being hydrolysed.

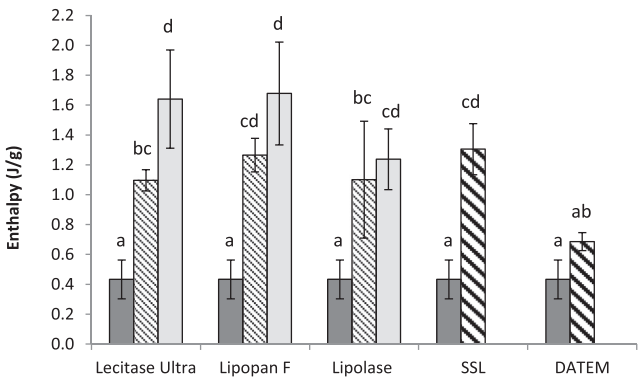

Fig. 1. DSC dissociation enthalpies (J/g dough sample) of amylose-lipid inclusion complexes upon heating of fermented dough samples without (▩) or with 0.5 (▧) or 5.0 (▩)mg EP/kg flour added lipase enzyme or 0.5% (▧) sodium stearoyl lactylate (SSL) or diacetyl tartaric acid ester of mono- or diacylglycerols (DATEM). Bars with the same letter are not significantly different ($\alpha = 0.05$).

SSL, a surfactant with a saturated FA tail (*i.e.* stearic acid), also induced formation of AM-L complexes (Fig. 1). Moreover, it did so to an extent similar to that of lipase addition. In contrast, DATEM, *i.e.* tartaric esters of mono- and diacylglycerols with mixed FA composition, slightly, but not significantly increased the enthalpy associated with dissociation of AM-L complexes (Fig. 1). This is in line with results from Colakoglu and Özkaya (2012). Thus, use of DATEM resulted in less AM-L complex formation than did lipase or SSL use. This can be attributed to its structure which probably contains unsaturated FA and/or two FA tails, which is not favourable for AM complexation because of its conformation.

3.2. Pasting, swelling and gelation behaviour of starch in wheat flour as affected by lipases and surfactants

Fig. 2 shows RVA profiles of control wheat flour suspensions and the same with added lipases or surfactants. The obtained profiles resemble those of wheat starch suspensions (Krog, 1973; Van Steertegem et al., 2013) showing that the changes observed are dominated by starch rather than by gluten protein transitions. Addition of Lecitase Ultra, Lipopan F, DATEM or SSL addition all significantly increased the peak and end viscosities of the wheat flour slurries. The effects were more pronounced with higher addition levels (Fig. 2). Besides this, pasting was significantly delayed (*i.e.* occurred at higher temperatures) upon addition of Lecitase Ultra (5.0 mg EP/kg flour), Lipopan F (5.0 mg EP/kg flour), DATEM (1.5% on flour basis) or SSL (0.5 and 1.5% on flour basis). In contrast, Lipolase addition did not influence the RVA profiles (*results not shown*).

Starch granule swelling has been mainly attributed to its AP fraction, while AM and lipids hinder swelling. The AM fraction (partly) leaches from the granules (Tester & Morrison, 1990). The present observations for DATEM and SSL are in line with findings by Krog (1973) that adding surfactants increase the starch pasting temperature. The author related this to formation of AM-L complexes. Pasting of starch granules in control flour occurred around 76.5 °C (Fig. 2). This, and the observation that AM-L complexes are formed at or below 60 °C (Karkalas et al., 1995), make it plausible that AM-L complexes can delay starch pasting. According to Eliasson (1985), formation of AM-L complexes upon addition of surfactants results in an insoluble film at the surface of the starch granules which impedes water transport into the starch granules. Putseys, Derde, et al., 2010, concluded from their study of the impact of glycerol monostearate on starch pasting in the RVA that adsorption of surfactant at the starch granule surface increases the pasting temperature. In this context, it is relevant that according to Eliasson, Carlson, Larsson, and Miezis (1981) lipid layers can easily be formed at the surface of starch granules.

SSL seemed more effective at postponing starch pasting than either DATEM or the tested lipases (Fig. 2). Remarkably, while Lipolase addition resulted in AM-L complex dissociation enthalpy readings which were similar to those of other wheat flour suspensions containing lipases or surfactants, it had no impact on starch swelling or pasting (Fig. 1). Hence, formation of AM-L complexes as such is no full-proof indicator for the swelling behaviour of starch granules present in wheat flour. The location of the lipid substrates also seems to be important. Lipolase mainly converts free TAG (Gerits, Pareyt, & Delcour, 2014) which are mostly located in spherosomes in wheat flour (Hargin, Morrison, & Fulcher, 1980). Lecitase Ultra and Lipopan F on the other hand mainly hydrolyse lipids in the bound lipid fraction, part of which is located at the starch granule surface. These lipids are mainly phospho- and galactolipids (Gerits et al., 2013) of amyloplast membrane remnants

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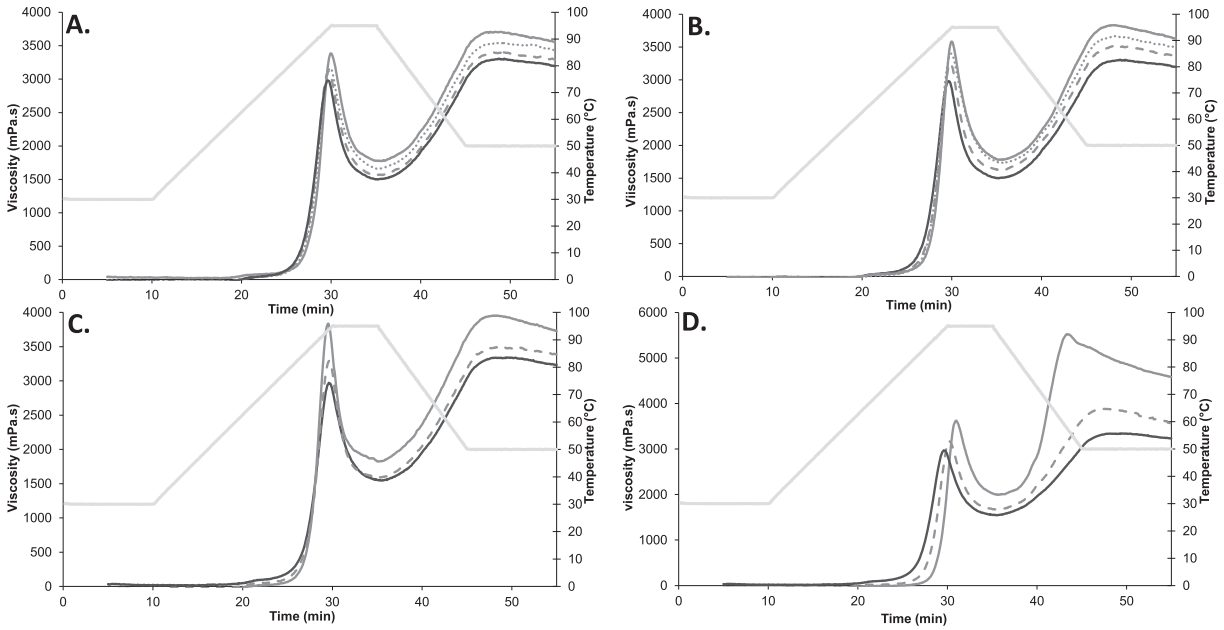

Fig. 2. RVA profiles of flour suspensions (12.0% dry matter) without (——) or with lipase addition [levels of 0.5 (– –), 1.0 (·····) and 5.0 (——) mg enzyme protein/kg flour] or surfactants [0.5% (– –) and 1.5% (——)]. (A) Lecitase Ultra, (B) Lipopan F, (C) diacetyl tartaric acid ester of mono- or diacylglycerols (DATEM), and (D) sodium stearoyl lactylate (SSL).

(Pauly, Pareyt, De Brier, & Delcour, 2014). The hydrolysis of these particular lipids into FFA and 'lyso' lipids more effectively impacted starch behaviour than did hydrolysis of lipids not present at the starch surface, as in the case of Lipolase. That DATEM delays starch pasting to higher temperatures without forming significant amounts of AM-L complexes lends further support for the view that AM-L complex formation is not necessarily predictive for altered starch pasting behaviour. It also contradicts the hypothesis that AM-L complex formation in itself is responsible for delaying starch pasting. Krog (1973) found that the effect of DATEM depends rather on the anionic polar head group than on the FA type (saturated or unsaturated) it contains. This would mean that DATEM, because its structure does not lend itself to AM-L complex formation, mainly acts by shielding the granule surface, and thereby impedes water absorption. Nevertheless, when AM-L complexes are formed, as in the case of lipase or SSL supplementation, they probably further influence pasting. However, whether the difference in pasting temperatures of flour suspensions containing either SSL or DATEM originates from the higher propensity of SSL to form AM-L complexes than that of DATEM (Fig. 1) or from SSL adhering more efficiently at the starch granule surface than DATEM, remains unclear at present.

Lipase hydrolysis products consist of a mixture of unsaturated and saturated FFA and 'lyso' lipids with saturated or unsaturated FA-tails. As described by Putseys, Lamberts, & Delcour, 2010, complexes of AM with unsaturated FA have lower thermal stability than those with saturated FA. Additionally, SSL and DATEM supplement the pool of endogenous polar lipids that already occur at the starch granule surface, whereas Lecitase Ultra and Lipopan F alter the composition of the starch surface associated lipid fraction. The more pronounced impact on pasting upon SSL addition than upon supplementation with Lecitase Ultra or Lipopan F probably is due to the higher stability of the AM-L complexes formed and/or the additional SSL covering the starch granule surface.

Whether or not the altered lipid composition at the starch granule surface also impacts starch pasting by acting directly on AP is still under debate and questionable. Van Steertegem et al. (2013) found hardly any impact of SSL and monoacylglycerols (MAG) on waxy maize starch pasting. However, Gudmundsson and Eliasson (1990) earlier demonstrated that when incubating 100% potato AP with MAG, the latter can directly hinder the formation of a three-dimensional AP structure and, thus, retrogradation. However, whether this is due to lipids forming complexes with the outer branches of AP is still unclear. Whatever be the case, if such complexes were to be formed, they cannot be detected by DSC. Also, the relatively short chain length of AP outer chains [*i.e.* DP 20-25] than of AM [*i.e.* average DP of 500 (Hizukuri, Takeda, & Yasuda, 1981)] may well hinder their interaction. Gudmundsson and Eliasson (1990) stated that, in the presence of AM, as in wheat flour [in which *ca.* 26% of wheat starch is AM (Lineback, 1984)], lipids more easily form complexes with AM than with AP.

The higher peak viscosity (Fig. 2) of suspensions containing Lecitase Ultra, Lipopan F or added surfactants under the experimental conditions implying a concentration regime above that of close packing C* can probably be attributed to the enzymatically formed or added lipids at the granule surface. This inhibits water uptake which, in turn, increases starch granule rigidity. The starch

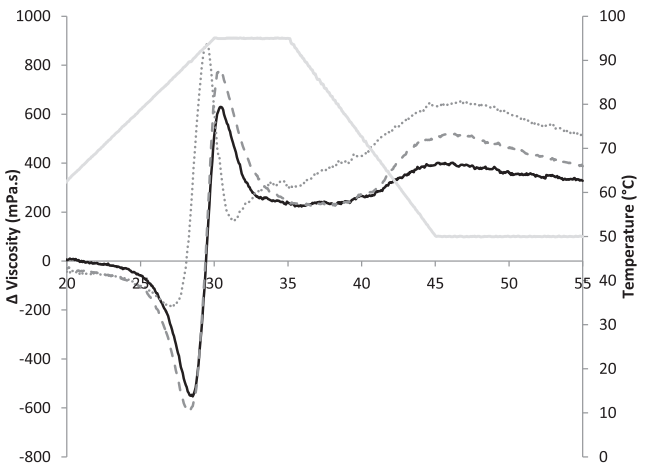

Fig. 3. Differences in RVA viscosities (mPa.s) between flour suspensions (12.0% dry matter) containing 5.0 mg EP/kg flour lipase enzyme [Lecitase Ultra (——) or Lipopan F (– –)] or 1.5% (on flour basis) DATEM (·····) and control flour suspensions.

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Table 1
Swelling power (SP, g/g) and carbohydrate leaching (CHL, %) of starch in wheat flour control or the same upon addition of 5.0 mg EP lipase/kg flour or 1.5% (flour basis) surfactant.

	45 °C		75 °C		95 °C	
	SP (g/g)	CHL (%)	SP (g/g)	CHL (%)	SP (g/g)	CHL (%)
Control	2.34 ± 0.04 b	3.11 ± 0.18 a	8.75 ± 0.16 a	6.55 ± 0.15 a	16.28 ± 0.48 a	31.18 ± 2.59 a
Lecitase Ultra	2.51 ± 0.02 a	3.33 ± 0.25 a	8.60 ± 0.10 ab	6.15 ± 0.07 ab	16.59 ± 0.32 a	30.17 ± 2.87 a
Lipolase	2.46 ± 0.07 ab	3.20 ± 0.08 a	8.78 ± 0.06 a	6.58 ± 0.18 a	15.64 ± 0.39 ab	30.17 ± 0.75 a
SSL	2.33 ± 0.03 b	3.07 ± 0.13 a	7.43 ± 0.29 c	5.03 ± 0.16 c	15.62 ± 0.24 ab	32.90 ± 1.09 a
DATEM	2.55 ± 0.07 a	3.05 ± 0.04 a	8.31 ± 0.03 b	5.92 ± 0.30 b	14.96 ± 0.96 b	31.07 ± 2.75 a

SSL, sodium stearoyl lactylate; DATEM; diacetyl tartaric acid esters of mono- or diacylglycerols.
Averages and standard deviations of three replicates are indicated. Values with the same letter in a same column are not significantly different ($\alpha = 0.05$).

granules then become more resistant to the applied shear. Once gelatinization has occurred in aqueous media, long stretched AM double helices and, in the longer run, also crystals thereof account for the stiffness of aqueous starch gels (Putseys, Gommes, Van Puyvelde, Delcour, & Goderis, 2011). According to Waterschoot, Gomand, Willebrords, Fierens, and Delcour (2014), the RVA end viscosity is the result of interplay between formation of an AM network and the presence of granule remnants. These remnants interact with the leached molecules, thereby reinforce the formed network and contribute to the paste viscosity. Additionally, Osman and Dix (1960) and, more recently, Putseys, Derde, et al. (2010) related the large viscosity increase around 70 °C upon cooling of starch suspensions to which MAG were added to the formation of AM-L complexes. Differences in RVA end viscosities of flour suspensions with added Lecitase Ultra, Lipopan F or SSL and control flour suspensions could therefore be ascribed to a different behaviour of the starch granule remnants during heating, to different properties of the obtained gels and/or to AM-L complex formation. Remarkably, only flour suspensions to which SSL had been added showed such large viscosity increase around 70 °C (Fig. 2D), whereas that observed upon addition of Lipopan F, Lecitase Ultra or DATEM was much smaller. However, plotting the differences in viscosities of these slurries relative to that of the control (Fig. 3) also revealed (small) viscosity increases around 70 °C during cooling. However, that the relative increases in viscosity around 70 °C between control flour suspensions and such suspensions with added Lecitase Ultra or Lipopan F were limited led us to conclude that the difference in end viscosity could not only be ascribed to AM-L complex formation but mainly to a different behaviour of the starch granule remnants during heating. Indeed, for Lecitase Ultra and Lipopan F, higher RVA end viscosities mainly resulted from increased peak viscosities which were mostly maintained during cooling (Fig. 2).

Additionally, SP and CHL upon addition of Lecitase Ultra, Lipolase, DATEM or SSL were determined at 45 °C, 75 °C and 95 °C (Table 1). These temperatures respectively correspond to temperatures below those of pasting, around pasting and the maximum temperature in the RVA. Because Lipopan F exerted effects similar to those of Lecitase Ultra, this enzyme was not included in the study. At 45 °C, CHL was similar for all samples and probably corresponded to the amount of soluble carbohydrates present in the wheat flour used (Table 1). As expected from the delayed pasting (Fig. 3), at 75 °C clear differences in granule rigidity appeared between the different samples. DATEM and SSL significantly, and Lecitase Ultra slightly (but not significantly) decreased SP and CHL. Eliasson (1985) also noticed a decrease in AM leaching and hypothesized that it was attributable to formation of AM-L complexes. However, as was the case for the pasting temperature, the present data upon addition of DATEM and Lipolase lend no support to this hypothesis. Indeed, DATEM hardly formed AM-L complexes (Fig. 1) but still significantly reduced CHL. Therefore, formation of AM-L complexes alone is not responsible for the decrease in SP and CHL. Indeed, a second important effect contributes to decreasing SP and CHL. This most logically is shielding of the granules by the lipophilic components. In contrast, at 95 °C all samples showed similar levels of CHL whereas SP significantly decreased upon addition of DATEM (Table 1). The lack of impact of the addition of Lecitase Ultra, Lipopan F or SSL on the SP and CHL at 95 °C could be ascribed to dissociation of AM-L complexes, which dissociate between 96 and 100 °C (Eliasson, 1985; Ghiasi, Hoseney, & Varriano-Marston, 1982). The lipophilic shield formed by DATEM, on the other hand, appeared more resistant to temperature increases. However, although significant, differences in SP at 95 °C were relatively smaller than at 75 °C, because of more pronounced swelling of all samples at the former temperature.

4. Conclusions

Under the experimental conditions of this work, Lecitase Ultra, Lipopan F, Lipolase and SSL all significantly induced AM-L complex formation. Such formation was independent of the lipid type and location. In literature, formation of AM-L complexes has been said to delay starch pasting and increase RVA peak and end viscosities. The present study revealed that although AM-L complex formation plays a role in wheat starch swelling and carbohydrate leaching, there is no causal relation between AM-L complex formation and wheat starch pasting behaviour. Indeed, Lipolase use induced AM-L complex formation but did not affect the swelling of wheat starch granules. In addition, while DATEM did not complex significant amounts of AM, it significantly impacted wheat starch swelling. It was concluded that both the location (*i.e.* in the gluten network or at the starch granule surface, as is the case for Lecitase Ultra and Lipopan F, or in spherosomes in the case of Lipolase) and type (*i.e.* mainly polar lipids by Lecitase Ultra and Lipopan F *vs.* mainly non-polar lipids in the case of Lipolase) of lipids are important for affecting starch swelling. Additionally, next to the complex forming ability of lipid(-like) components, their ability to shield starch granules as a lipophilic layer can also postpone starch pasting because of delayed water absorption. The increased RVA peak viscosities upon addition of Lecitase Ultra, Lipopan F or surfactants can be ascribed to increased starch granule rigidity. At concentrations exceeding those of C*, the higher RVA end viscosities upon addition of DATEM, Lecitase Ultra or Lipopan F Ultra mainly result from starch granule higher rigidity during heating. However, for samples containing SSL, a large additional increase around 70 °C occurred which was attributed to AM-L complex formation.

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